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Physico-chemical standardisation of Hansraj (*Adiantum capillus-Veneris)*

Md Nazim Ali[1*], Dr Mohd. Aslam[2], Rizwana Khatoon[3], Dr. Mohd. Asif[4], Shahid Shah Chaudhary[5]

1. M D Scholar, Department of IlmulAdvia, SUMER, Jamia Hamdard
2. Professor, Department of IlmulAdvia, SUMER, Jamia Hamdard
3. M D Scholar, Department of IlmulAdvia, SUMER, Jamia Hamdard
4. Assist. Professor, Department of IlmulAdvia, SUMER, Jamia Hamdard
5. Assist. Professor, Department of IlmulAdvia, SUMER, Jamia Hamdard

ABSTRACT

Ethno-botanical use and traditional knowledge of plants have been usually recognized all over the world. The outstanding characteristics of extracts obtained from this drug have been technically studied, attributing the main biological activity to its tannin and flavonoid content. Recent study about this drug *Adiantum capillus-veneris* Linn requires pharmacognostical evidences to develop quality-control methods for raw materials and extracts produced with this plant drug. In this research article macro and micro-scopic studies were proven the authentication of the real drug in which adulteration is allowed in commercial samples of this plant material. The parameters which were mention in this study all are according to WHO guidelines and Indian Pharmacopoeia (IP). The morphological characteristics present in this article can be useful for quick identification of the drug, particularly useful in the case of powdered drug. Physicochemical parameters such as total ash, water soluble ash, acid insoluble ash, loss on drying, percentage of foreign matter and extractive values were determined. Preliminary phytochemical screening in different solvents showed the presence of flavonoids, terpenoids, fats, tannins and phenolic compounds. The various phytochemicals presence in the drug is confirmed by thin layer chromatography (TLC) profile. The study can serve as a valuable source of information and provide suitable standards for the presence of various phytochemicals. The results extracted from this study such as identification, phytochemical investigation etc. could be helpful in future research and uses.

Keywords: Ethno-botanical use , Hansraj, flavonoids

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***Address for Correspondence:**

Md Nazim Ali, M D Scholar, Department of IlmulAdvia, SUMER, Jamia Hamdard

INTRODUCTION

From the ancient time, natural herbs have been used as medicines to get rid of various kinds of diseases, Medicinal plant have played very important role in World health. In spite of the significant advances in allopathic medicines in recent decades, medicinal plant still make a predominant contribution to health care. Herbal products have been most believable source of medicine [1]. India is big field for development of herbal medicine like Unani, Ayurveda, Siddha, Homoeopathy and other natural herbs based health science, because so many types herbs is found in our country and some drugs are unexplored so this field is in need of more research (Preclinical and clinical). *Hansraj (Adiantum capillus-veneris Linn.*) is one of the most common species with potential importance for medicinal and nutritive purpose [2]. *Hansraj (Adiantum capillus-veneris Linn.*) is a herbal drug kind of medicinal and ornamental fern belonging to the family Pteridaceae widely distributed throughout the world [3] [4] It is a delicate graceful fern, small rhizomatous, erect and perennial herb up to 30 cm tall with long polishe black stripes [5] [6] [7].

Standardization of herbal medicine means confirmation of its identity, quality and purity of all phases of its cycle i.e. shelf life, storage, distribution and uses by different parameters [8]. World Health Organization currently promotes and recommends natural/herbal medicines in national Health care program because these drugs are available easily and the cost of these drugs are very cheap so herbal drug are affordable for every person and these drugs are comparatively safe and the people have faith in such remedies. Plant materials like gums, leaf, seeds, bark etc. are used throughout the world included developed country as home remedies over the counter drug products and raw materials for the pharmaceutical industry and represent substantial proportion of the overall medicinal market. It is therefore essential to establish internationally recognized guidelines for assessing their quality. The world Health Assembly in resolutions WHA 31.33 (1978), WHA 40.33 (1987) and WHA 42.43 (1989) has emphasized the

need to ensure the quality of medicinal plant products by using advanced control techniques and applying suitable standards.

Availability of genuine drug is mainly affected by adulteration, substitution and lack of skilled personal. Quality and freshness is defined as the purity of a drug which is ascertained by identity, chemical, physical or biological properties. For the conservation of quality of natural products, the process of control of quality is paramount importance. Quality control of herbs is based on three pharmacopoeial aspect including identification of herb, purity and free from adulteration of herb, assay of the active constituent of herb [9].

Standardization is the process of determination of value of herbal plant like qualitative and quantitative values of natural/herbal medicine by prescribing a set of standards or inherent characteristics and constant parameters that give assurance of quality, experimentation and observation lead to the setting of specific standard for that particular herbal/natural medicine [10]

MATERIALS AND METHODS

Materials Used

Chemicals & reagents:

Acetic acid	S D Fine chemical Pvt. Ltd. (Mumbai India)
Aluminum Chloride	Central drug house Pvt. Ltd. (New Delhi, India)
Butylated hydroxyl toluene	E Merck Pvt. Ltd. (Mumbai India)
Benzoic acid (AR)	E. Merck (India) Ltd., Mumbai
Carboxyl methyl cellulose (LR)	Central Drug House (CDH) Ltd., Bombay
Chloroform (LR)	Central Drug House (CDH) Ltd., Bombay
Creatinine (AR)	Central Drug House (CDH) Ltd., Bombay
Diethyl ether	S D fine chemical Pvt. Ltd. (Mumbai. India)
EDTA	E Merck Pvt. Ltd. (Mumbai India)
Dragendroff's reagent	E Merck Pvt. Ltd. (Mumbai India)
Diacetyl monoxime (AR)	Central Drug House (CDH) Ltd., Bombay
Ether (LR)	Central Drug House (CDH) Ltd., Bombay
Ethyl alcohol or Ethanol	S D fine chemical Pvt. Ltd. (Mumbai, India)
Ferric chloride (AR)	Central Drug House (CDH) Ltd., Bombay
Formalin	SD fine Chem. Ltd., Bombay
Folin Ciocalteu	Thomas Baker Pvt. Ltd. (Mumbai, India)
Galic Acid	S D fine chemical Ltd. (Mumbai, India
Gentamicin sulphate	German Remedies Ltd., Bombay
Hematoxylin-eosin	S D fine chemical Pvt. Ltd (Mumbai, India)
Hydrochloric acid (LR)	SD fine Chem. Ltd., Bombay
Iodine soln.	Central drug house Pvt. Ltd. (New Delhi, India)
Magnesium	Central drug house Pvt. Ltd. (New Delhi, India)
Mayer's reagent	Central drug house Pvt. Ltd. (New Delhi, India)
Molisch's reagent	S D fine chemical Pvt. Ltd. (Mumbai, India)
Methanol (LR)	Central Drug House (CDH) Ltd., Bombay
NADPH	Sigma chemicals Co. (St. Louis, MO, USA)
Orthoposphoric acid (AR)	SD fine Chem. Ltd., Bombay
Picric acid (AR)	Central Drug House (CDH) Ltd., Bombay
Petroleum ether (60-80) (LR)	E. Merck (India) Ltd., Mumbai
Reduced glutathione (GSH)	Sigma chemicals Co. (St. Louis, MO, USA)
Sodium hydroxide (AR)	Central Drug House (CDH) Ltd., Bombay
Sodium tungstate hydrated (LR)	Central Drug House (CDH) Ltd., Bombay
Sulphuric acid (AR)	SD fine Chem. Ltd., Bombay

Thiosemicarbazide (AR)	Central Drug House (CDH) Ltd., Bombay
Urea (AR)	Central Drug House (CDH) Ltd., Bombay

Double distilled water is used wherever required.

LR=Laboratory reagent

AR= Analytical reagent

(b) Instruments and equipment:

Analytical Balance, Dhona 200D	Dhona Instruments (Pvt.) Ltd.
Centrifuge machine, model C-854	scientific systems, New Delhi
UV-VIS Spectrophotometer, model UV-1201	Schimadzu, japan.
Micropipettes	Tarson, India.

Plant's materials:

The drug, Hansraj (*Adiantum Capillus-veneris*) were purchased from Shamshi Dawakhana, Ballimaran Old Delhi 110006. The identity of the purchased drugs were established as crude dried drug of Hansraj by the scientist working at NISCAIR (national institute of science communication and information Resources), Dr. K.S. Krishnan Marg, Pusa Gate, New Delhi, 110012. Voucher specimen of drug coded as (NISCAIR/RHMD/Consult/2017/3076-25) for Hansraj (*Adiantum capillus-veneris*) respectively, have been retained and deposited in the department of Ilmul Advia, School of Unani medicine Education & Research, Jamia Hamdard, New Delhi 62.

Parameters of standardization:

Sterilization of *Hansraj (Adiantum capillus-veneris)* was carried out using following parameters.

1. Morphology / Organoleptic properties
2. Microscopy
3. Foreign Matter
4. Physicochemical Parameters and Phytochemical Evaluation [11] [12] [13]

i. Loss on Drying (LOD) at 105˚C

ii. Ash value

a) Total ash

b) Acid insoluble ash

c) Water soluble ash

iii. Determination of pH:

a) 5% solution

b) 10% solution

iv. Extractive values

a. Alcohol soluble extractive values

b. Water soluble extractive values

v. Fluorescence analysis

vi. Powdered drug reaction with different reagents

vii. Estimation of Total Phenolic

viii. Phytochemical investigation

ix. TLC Profile

Morphological Characters

In many cases, general appearance of the herbal plant and any part of the plant which is used in disease is similar to other related species. Detailed study of the morphological characters can be helpful in differentiating them from the associated drug. The microscopy of a drug includes its visual appearance to the naked eye. It depends to a large extent on the part of the plant from which the drug is acquired [14]

Determination of foreign matter

Herbal drug should be made from the described part of the plant and be unattached of other parts of the same plant or other plant. They should be completely free from moulds or insects, including excreta and visible contaminants such as sand and stones, poisonous and harmful foreign matter and chemical residues [15] [16] [17]. Foreign matter can easily determine by macroscopic examination even though, microscopic examination is only required in certain special cases (for example, starch deliberately added to "dilute" the plant material). In any way if foreign matter is still persist then for example, of a chemical residue, TLC is often needed to detect the contaminants [18] [19].s

Weight 250 g of the drug was examined and spread it out in a thin layer. Examined for foreign matter by inspection with the unaided eye or by use of a lense (6 ×). Separate foreign matter and weight it and calculated the percentage present.

Loss of weight on drying

The natural drug contain variable limit of moisture. When untreated herbs are sold in the market with the confirmation assay for active constituent must be calculated on the behalf of moisture free drugs or should be calculated taking in to consideration the percentage of water therein. This parameter is used to determine the amount of moisture present in a particular sample.

Methods:

The powder drug 5 g sample was placed on a tarred petri dish. The tarred petri dish was dried at 105 °C for 6 hour and weighed. The process was continued until two successive reading matched each other or the difference between two successive weighing was not more than 0.25 % of the constant weight.

pH of crude drug

Determination of pH of a drug by a drug solution is very important because after confirmation we can say that drug is basic, acidic or neutral in nature.

pH 1% solution

1 gm drug was and dissolved in 100 ml of distilled water and filtrate was checked with standard glass electrode [20]

pH 10 % solution

10 gm drug was taken and dissolved in 100 ml of distilled water and filtered; pH of filtrate was checked with standard glass electrode**Invalid source specified.Invalid source specified.**

Extractive Values

The quantity of an extract that a drug yields in a specific solvent is often an approximate measure of the amount of certain types of constituent that accommodate in drug. The drug should be extracted with different type of solvent in order their increasing polarity to get the correct and reliable values. Mostly petroleum ether, alcohol, and water extractives are taken into consideration for fixing the standard of drug. The extraction of petroleum ether contains fixed oil, resins and volatile substances, but when 105 °C temperature gives to extract until constant weight, the volatile substance of the extract volatilized only resin, coloring matter and fixed oil remaining in extract. Alcohol can dissolve almost all the substances, but alcohol generally used for determining the extractive index for those drugs, which contain glycosides, resins, alkaloids etc. water is used for the drugs containing water soluble substances as chief constituents. The values of extractive substance were determined according to the method described in pharmacopoeia [15] [21].

Cold extraction

The air-dried coarse powder of drug (10 g) was infused with different solvents separately (petroleum ether, chloroform, methanol and water) of volume 100 ml in a closed flask for 24 hour, shaking regularly during 6 hours and allowed to stand for 24 hours and filtered quickly, taking precaution at the time of this processing against loss of solvent, the filtrate evaporated to dryness in a tarred flat bottom dish and dried at 105°C, to constant weights and weighed**.**

Hot extraction

The powdered drug (10 g) was filled in a soxhlet apparatus individually for every solvent i.e. petroleum ether, chloroform, methanol and water. Every extract was vaporize to dryness and their constant weight were recorded**.**

Ash Value

Ash value is very important property of a drug. This parameter use for identification of the drug adulteration as well as establish the quality and purity of the drug. Total ash is designed to total amount of remaining material produced after complete incineration of the drug at the temperature 450 °C to remove all the carbons which was present in the drug. At higher temperature, the alkali chloride may be volatile and may be destroy by this process. The total ash usually containing of carbonate, phosphate, silicates and silica which have both type of ash physiological and non-physiological ash. Physiological ash which is derived from the plant tissue itself and non-physiological ash which is the precipitate of the adhering material to the plant such as sand and soil [22] [15]

Method

The air dried crude drug (2g) was weighed in a crucible and was incinerated at the high temperature (450°C) until free from carbon. After that wait for normal temperature. The percentage of ash with reference to air-dried drug was calculated [21]

Acid insoluble ash

Ash insoluble in HCl (hydrochloric acid) is the remnant obtained after extracting the sulfated or total ash with HCl;

Method

The total ash was boiled with 25 ml of 2M HCl for 5 min. The insoluble matter was deposit in the base of crucible or on an ash less filter paper (Whatman filter paper) it was then washed with hot water, cooled in desiccator and was weighed. The percentage of acid insoluble ash with reference to the air-dried drug was calculated.

Water soluble ash

Water soluble ash is the part of total ash which is soluble in water. It is good indicator of either previous extraction of the water soluble salts in the drug or incorrect preparations. Thus, it is the difference in weight between the total ash and the residue obtained after treatment of total ash with water.

Method

Ash was dissolved in 25 ml distilled water and that which is not soluble in water collected on whatsman filter paper (ash less filter paper), filter paper with its content ignited at 450°C to a constant weight. By subtracting the weight of insoluble part from that of the ash, the weight of the soluble part of ash was obtained.

1. Fluorescence analysis

Many herbal drug gives fluorescence when cut surface or powder is exposed to UV light and this can help in their identification method

Method

Powdered drug was treated with different reagents and examined under UV light (255 and 366 nm). Different reagents showed different colors of drug.

2. Powdered drug reaction with different reagent

Powdered drug's chemical test with different reagents were performed according to method described by Sama (23).Generally these test based on color indication of powdered drug with specific substance which is mention in results.

2. Estimation of total phenolic

In organic chemistry, phenols, sometimes called phenolic, are a class of chemical compound consisting of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. The simplest of the class is phenol, which is also called carbolic acid.

Phenols probably constitute the largest group of plant secondary metabolites widespread in nature, and to be found in most classes of natural compounds having aromatic moieties. Phenols are important constituents of some medicinal plants and in the food industry, they are utilized as coloring agents, flavorings, aromatizes and antioxidants [24]

3. Phytochemical investigation

Every plant may considered as a biosynthetic laboratory for a multiple of compounds like alkaloids, glycosides, volatiles oil, tannins, etc that exert physiological effects. These compounds are responsible for therapeutic effect

are usually the secondary metabolites. The plant material was subjected to preliminary phytochemical screening for the detection of various plant constituents. [14]

Test for saponins:

A 250 mg. (amount of extract is not fix) of the aqueous methanolic extract was taken in a test tube and shake vigorously with a 20 mg. sodium bicarbonate ($NaHCO_3$) and water. A stable, characteristic honeycomb like froth was obtained indicating the saponins is positive in giving drug.

Test for tannins:

Tannin is the derivative of the gallic acid. It is a yellowish or brownish bitter taste organic substance present in barks and other plant tissues.

A few mg. of aqueous methanolic extract was taken separately in water, warmed and filtered. Test were carried out with the filtrate using following reagents.

Ferric chloride reagent

A 5% w/v solution of ferric chloride in 90 % alcohol was prepared. Few drop of this solution were added to a little of the above filtrate. Dark green or deep blue color was indicating the presence of tannins.

Lead acetate test

A 10 % w/v solution of basic lead acetate in distilled water was added to the test filtrate. If precipitate is obtained, tannins are present.

Potassium dichromate test

Addition of solution of potassium dichromate in filtrate, dark color is developed, tannins are present.

Test for Flavonoids (Shinoda test)

In this test, a small quantity of aqueous methanolic extract was dissolved in 5 ml ethanol (95% v/v) and reacted with few drops of conc. Hcl and 0.5 mg of magnesium metal. The pink, crimson or magenta color is developed within a minute if flavonoids are present.

Test for Proteins:

Biuret test

Some amount (mg) of the aqueous methanolic extract was taken in water and 1 ml of 4 % NaOH was added to it. A drop of 1 % solution of copper sulfate followed this violet or pink color is formed if proteins are present.

Xanthoproteic test

This method can be used to determine the amount of protein in giving sample.

A few mg of aqueous methanolic extract was taken with 2 ml of water and 0.5 ml of concentrated nitric acid was added to it. Yellow color is obtained if proteins are present.

4. Thin layer chromatography (TLC profile)

TLC is a special technique for characterization of a plant drug [25]. Different fractions of the drug viz petroleum ether fraction, chloroform fraction and methanolic fractions were subjected to TLC and the TLC fingerprints were recorded.

Thin layer chromatography (TLC) assay was conducted using aluminum sheets of silica gel (TLC silica gel 60 F_{254} Merck). Extract of plant material was dissolved in a volatile solvent to produce a very dilute solution. Little amount of extract solution was applied on the plate or stationary phase (The layer adsorbent known as stationary phase). After the sample has been applied on the plate, a solvent or solvent mixture (known as mobile phase) is drawn up the plate via capillary action. The plate was developed in the developing chamber containing shallow pool of solvent just below the level of at which the sample was applied. The solvent was drawn up through the particles on the plate through the capillary action and as the solvent moves over mixture each compound dissolved in the solvent and move up the plate. When the solvent front moved to upper end of adsorbent, the plate was removed from the TLC developing chamber, solvent was allowed to evaporate and the solvent front was marked. The plate was placed in iodine chamber and then visualized under UV light in UV chamber. Measurement of Rf was determined and value was showed as decimal fraction and was calculated by dividing the distance the compound travelled from the original position by the distance the solvent travellrd from the original position (solvent front) [26]

OBSERVATIONS AND RESULTS

01. Morphological Characters.

Hansraj *(Adiantum capillus-veneris)* is looking like coriander herb. Stripes suberect, shining dark brown hairless petioles. The fronds are badly broken but a few intact branches show that it is 3-4 pinnate. The segments (Pinnae) are bright green, shortly petiolulate, glabrous, 6-9 mm long and 3-5 mm broad in the middle. The blade has prominent non reticulate branching veins. The blade is simple, not cleft or lobed. The upper margin is rounded with dentate margin while the lower part is simple (cuneate). Some fertile segments are also present these are also un-lobed, only some slightly bi-lobed. It is tasteless and gives a slightly aromatic smell.

Parameters	Leaf	Stem
Color	Bright green	Dark purplish to black
Shape	Wedge or fan shaped	Sub erect
Odour	Fragrant smell	Aromatic
Length	1.2-2.0 cm	10-20 cm
Breadth	1.25-2.5 cm	2 mm in diameter
Base	Cuneate	Scaly
Texture	Thin	Smooth
Taste	Slightly bitter	Slightly bitter
Touch	Smooth	Soft

1. Determination Of Foreign Matter:

250 gm. Drug (three samples) was taken for examine and remove all unused parts remove from the drug and weight them. The weight of the drug after removal of foreign matter was 225 gm., 223.80 gm., 224.00 gm. that was the 10.29 (Mean value) of total drug.

Foreign matter analysis of Hansraj *(Adiantum capillus-veneris)*

S.NO.	Weight of drug (g)	Weight of foreign matter	Foreign matter %	Mean ± SEM
1.	250	25.0	10	
2.	250	26.20	10.48	
3.	250	26.00	10.40	10.29 ± 0.14

Moisture Content:

Wt of drug(gm)	Wt of empty China dish (gm)	Wt of empty China dish (gm) + Wt of drug (gm)	Wt of empty China dish (gm) + Wt of drug (gm) after drying	Drug %	Mean ±SEM
5	50.40	55.40	54.90	10	
5	50.42	55.42	54.96	9.2	
5	50.39	55.39	54.98	8.2	9.13 ± 0.52

pH of powdered drug

10% - 5.76

5% - 5.78

Extractive Values: (Hot and cold extraction)

Individual Cold extraction values

Petroleum ether extracts (Cold)

S.NO.	Wt. of drug	Wt. of China dish	Wt. of China dish+ Wt. of extract	Wt. of extract	Extractive value (%)	Mean ± SEM
1.	10	116.40	117.28	0.88	8.8	
2.	10	103.35	104.25	0.90	9.0	8.83 ± 0.088
3.	10	120.55	121.42	0.87	8.7	

Chloroform extracts (Cold)

S.NO.	Wt. of drug	Wt. of China dish	Wt. of China dish+ Wt. of extract	Wt. of extract	Extractive value	Mean ± SEM
1.	10	104.50	105.54	1.04	10.4	
2.	10	100.50	101.70	1.20	12	11.13 ± 0.46
3.	10	98.00	99.10	1.10	11	

Methanol extracts (Cold)

S.NO.	Wt. of drug	Wt. of China dish	Wt. of China dish+ Wt. of extract	Wt. of extract	Extractive value (%)	Mean ± SEM
1.	10	90.05	90.79	0.74	7.4	
2.	10	91.60	92.36	0.76	7.6	7.56 ± 0.088
3.	10	88.20	88.97	0.77	7.7	

Aqueous extract

S.NO.	Wt. of drug	Wt. of China dish	Wt. of China dish+ Wt. of extract	Wt. of extract	Extractive value (%)	Mean ± SEM
1.	10	90.00	90.25	0.25	2.5	
2.	10	92.40	92.60	0.20	2	2.5 ± 0.28
3.	10	88.00	88.30	0.30	3	

5. Successive Extraction: (Hot extraction)

Petroleum ether extracts

S.NO.	Weight of drug	Weight of china dish	Weight of China dish +Wt. of Extract (g)	Weight of extract	Extractive value	Mean ±SEM
1.	50	43.42	45.38	1.96	3.92	3.92 ± 0.011
2.	50	42.40	44.40	1.95	3.90	
3.	50	44.00	45.97	1.97	3.94	

Chloroform extract

S.NO.	Weight of drug	Weight of china dish	Weight of China dish +Wt. of Extract (g)	Weight of extract	Extractive value	Mean ±SEM
1.	50	45.146	45.257	0.111	0.222	0.225 ± 0.0017
2.	50	44.237	44.351	0.114	0.228	
3.	50	44.464	44.577	0.113	0.226	

Alcoholic extract

S.NO.	Weight of drug	Weight of china dish	Weight of China dish +Wt. of Extract (g)	Weight of extract	Extractive value	Mean ±SEM
1.	50	46.764	53.672	6.908	13.816	13.717 ± 0.057
2.	50	45.444	52.252	6.808	13.616	
3.	50	46.664	53.524	6.860	13.720	

Aqueous extract

S.NO.	Weight of drug	Weight of china dish	Weight of China dish +Wt. of Extract (g)	Weight of extract	Extractive value	Mean ±SEM
1.	50	45.382	55.222	9.84	19.68	19.61 ± 0.035
2.	50	44.287	54.087	9.80	19.60	
3.	50	45.291	55.071	9.78	19.56	

6. Ash Values

Total Ash

S.NO.	Wt. of drug	Wt. of crucible	Wt. of crucible + wt. of ash (g)	Wt. of crucible after ignition (g)	Wt. of ash	Total ash (%)	Mean ± SEM
1.	5	45.57	50.57	45.95	0.38	7.6	8.26 ± 0.35
2.	5	45.00	50.00	45.44	0.44	8.8	
3.	5	48.54	53.54	48.96	0.42	8.4	

Acid insoluble ash

S.NO.	Wt. of drug	Wt. of Crucible	Wt. of Crucible+ Wt. of ash	Wt. of Crucible after ignition (g)	Wt. of ash	Total ash	Mean ± SEM
1.	5	45.54	50.54	45.71	0.17	3.4	3.33 ± 0.066
2.	5	48.30	53.30	48.47	0.17	3.4	
3.	5	45.00	50.00	50.16	0.16	3.2	

Water soluble ash

S.NO.	Wt. of drug	Wt. of Crucible	Wt. of Crucible+ Wt. of drug	Wt. of Crucible after ignition (g)	Wt. of ash	Total ash	Mean ± SEM
1.	5	33.76	38.76	34.16	0.40	8.0	8.33 ± 0.176
2.	5	35.89	40.89	36.31	0.42	8.4	
3.	5	36.00	41.00	36.43	0.43	8.6	

7. Fluorescent Analysis

S.NO.	TREATMENT	DAY LIGHT	UV LIGHT (254 nm)	UV LIGHT (366 nm)
1.	Powder + Pet. Ether	Transparent	Colorless	Light green
2.	Powder +Benzene	Green	Violet	White
3.	Acetone	Light Green	Light Brown	Green
4.	Ethyl Acetate	Light Green	Green	Violet
5.	Chloroform	Greenish Yellow	Light Brown	Light Black
6.	Methanol	Dark Green	Orange	Light Yellow
7.	Water	Straw colored	Colorless	Transparent
8.	Dil. Hcl	Brown	Colorless	Light Green
9.	Dil. HNO_3	Reddish	Light Green	Green
10.	Dil. H_2SO_4	Black	Colorless	White

8. Powdered drug Reaction with different reagents:

TREATMENT	OBSERVATION
Conc. HCL	Dark brown
Con. HNO_3	Reddish
Conc. H2SO4	Black
Iodine Solution	Iodine color
Glacial Acetic Acid	Dark Green
Powder as such	Greenish brown

9. Estimation of total phenolics

Wt. of drug	Wt. of empty petri dish	Wt. of petri dish (g) + Wt. of phenolic contant	Total phenolic content (gm)	Phenolic %
5 gm	47.180	47.329	0.149	2.98%
5 gm	47.219	47.370	0.151	3.02%
5 gm	47.170	47.319	0.149	2.98%

10. Results of Preliminary Phytochemical Tests of *Adiantum capillus-veneris*:

S.NO.	TEST	POWDERED DRUG
01	**TEST FOR SAPONINS** Foam Test	**Negative**
02	**TEST FOR TANNINS** Ferric chloride reagent Lead acetate test Potassium dichromate test	 **Positive** **Positive** **Positive**
03	**TEST FOR FLAVANOIDS** Shinoda Test	 **Positive**
04	**TEST FOR PROTIENS** Biuret Test Xanthoproteic test	 **Positive** **Positive**

TLC Data of Hansraj (*Adiantum Capillus-Veneris)*

S. NO.	Extract	Solvent system	Rf value in iodine chamber	No. of Spots
01	Chloroform	Chloroform: Ethyl acetate (8:2)	0.646 0.507 0.461 0.338 0.169 0.123	06
02	Petroleum ether	Benzene: Ethyl Acetate (9:1)	0.579 0.362 0.202 0.0579	04
03	Methanol	Chloroform: Ethyl Acetate (9:1)	0.272 0.218 0.145 0.090 0.0363	05

CONCLUSION

In this investigation various standardizations parameters such as macroscopy, microscopy, physicochemical constants, preliminary phytochemical investigation and TLC profiles of sequential extraction of sample in petroleum ether, chloroform and methanol extracts were studied, which are being reported for the first time in this plant, could be helpful in authentication and preparation of a suitable monograph for the proper identification of whole plant of *Adiantum capillus-veneris* Linn

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